Clean Copy of Paragraph, Page 1, after the title

CROSS REFERENCE

A1

This application is a continuation of U.S. Patent Application having Serial No. 09/203,679, filed December 1, 1998.

Clean copy of paragraph beginning at page 5, line 1:

A2

The Type II callus is then regenerated into plants. "Water tower" structures are generally in evidence as soon as callus is initiated from immature embryos. The desired Type II callus is cultured on solid medium to regenerate plants. The Type II callus is then regenerated into plants. Tissue containing a high frequency of "water tower" embryos structures is selected from the callus initiated from normal and "infected" immature embryos. This tissue is desirable since it allows for ready regeneration of plants. This desired Type II callus is cultured on solid medium to regenerate plants.

Clean Copy of paragraph beginning at page 11, line 13:

A3

Actively growing Type II callus is selected from the clonal tissue with the objective to obtain a high frequency of "water tower" embryo structures in the cultures. The tissue containing the "water tower" embryo structures is cultured on a solid medium to mature the embryos. Maturing embryos are transferred to solid medium to further the maturation and to induce germination. Germinating embryos are transferred to solid medium for the promotion of further root and shoot development prior to final transfer to soil. The solid medium may contain any conventional salt and vitamin mixture, such as MS salts with or without MS vitamins or other vitamins, N6 salts with or without N6 vitamins or other vitamins and the like. Methods for plant regeneration are known in the art and preferred methods are provided by Kamo et al. (1985), West et al. (1993), and Duncan et al. (1985).

Clean Copy of paragraph beginning at page 14, line 19:

Agrobacterium strain LBA 4404 harboring "super binary" vectors as described in U.S. patent Hei and Komari (1997) was used in corn transformation experiments. Vectors with a *bar* expression cassette from pBARGUS (Fromm et. al., 1990) were used to generate resistance to the herbicide bialaphos, and a *gus* expression cassette from pIG221 (Ohta et al., 1990) was used to produce Gus expression for transient assays. The *gus* expression cassette contains an intron in the N-terminal region of the *gus* gene which prevents expression in bacteria, but upon expression in plant cells the intron is spliced out and Gus activity is achieved (Ohta et al., 1990; Ishida et al., 1996). *Agrobacterium* containing "super binary" vectors were stored in glycerol stocks using acidified glycerol. Glycerol was acidified by adding 15 drops of 1M HCl to one liter of glycerol (Sigma G-9012). Final glycerol concentration of stocks was 15 to 20% and stocks were frozen at minus 86° C. When glycerol stocks were used as the source for transformation experiments, *Agrobacterium* was made ready for transformation experiments by removing a few flakes of frozen culture with a sterile loop, streaking it out on YP medium (5 g/l yeast extract, 10 g/l peptone, 5 g/l NaCl, and 15 g/l agar) containing 50 mg/l spectinomycin, and incubating it for one or two days at 28°C. When glycerol stocks were not used as the source, *Agrobacterium* maintained on YP plus spectinomycin at 4°C was used to initiate new cultures of *Agrobacterium* that were grown as described above.

Clean Copy of paragraph beginning at page 15, line 3:

Please replace the paragraph beginning at page 15, line 3, with the following rewritten paragraph:

Co-cultivation of the immature embryos and *Agrobacterium* cells in plant transformation work has been routinely performed at 25° C. Observations by Fullner et al. (1996) suggested that better results might be expected at lower temperatures. This was confirmed by Dillen et al. (1997) for transformation of tobacco. We therefore tested 19° C as a co-cultivation temperature for corn. Co-cultivating at 19° C is clearly superior as indicated by transient expression of the gus gene. Subsequently, all experiments were carried out at a co-cultivation temperature of 19° C. The protocol of Hei and Komari (1997) utilizes the corn inbred line A188 and hybrids with A188. No success was reported with other inbreds (Ishida et al., 1996). Their approach was tried with Stine 963 and was not successful. Cultured immature embryos of Stine 963 treated with *Agrobacterium* after Hei and Komari, and Ishida et al produced no transformed clones. The following modifications were then tried:

Clean copy of paragraph beginning at page 18, line 9:

(a) Actively growing Type II callus was selected from clonal tissue, with the objective of obtaining a high frequency of so-called 'water tower' embryo structures in the cultures.

Clean copy of new claims 35-42

35. (NEW) A method for transforming a line of corn comprising the steps of:

(a) co-cultivating an immature embryo from said line at a temperature of about 15°C to about 22°C with *Agrobacterium* capable of transferring at least one gene to tissue of said line to produce an infected embryo;

(b) culturing the infected embryo on a medium comprising an antibiotic;

(c) culturing the resulting tissue on a medium comprising a selective agent;

(d) selecting transformed tissue comprising growing Type II callus; and

(e) regenerating transgenic plants from said Type II callus.

36. (NEW) The method of claim 35, wherein said temperature is about 19°C.

37. (NEW) A method for transforming a line of corn comprising the steps of:

(a) co-cultivating an immature embryo from said line with *Agrobacterium* capable of transferring at least one gene to tissue of said line to produce an infected embryo, wherein said *Agrobacterium* is taken from *Agrobacterium* about 0.5 to about 5 days after rescue from frozen glycerol stocks;

(b) culturing the infected embryo to initiate callus on a medium comprising an antibiotic;

(c) culturing the resulting callus tissue on a medium comprising a selective agent;

(d) selecting transformed callus tissue comprising growing Type II callus; and

(e) regenerating transgenic plants from said growing Type II callus.

38. (NEW) A method for transforming a line of corn comprising the steps of:

(a) co-cultivating an immature embryo from said line with *Agrobacterium* capable of transferring at least one gene to tissue of said line to produce an infected embryo;

(b) culturing the infected embryo to initiate callus on a medium comprising an antibiotic and a monosaccharide sugar;

(c) culturing the resulting callus tissue on a medium comprising a selective agent;

(d) selecting transformed callus tissue comprising growing Type II callus; and

(e) regenerating transgenic plants from said growing Type II callus.

39. (NEW) The method of claim 38, wherein said monosaccharide sugar is selected from the group consisting of glucose, maltose, lactose, sorbitol and mannitol.

40. (NEW) The method of claim 38, wherein said monosaccharide sugar is glucose.

41. (NEW) The method of claim 38, wherein the concentration of said monosaccharide sugar is from 5 g/L to 30 g/L.

42. (NEW) A method for transforming a line of corn using *Agrobacterium* comprising the steps of:

(a) initiating co-cultivation of an immature embryo from said line with *Agrobacterium* capable of transferring at least one gene to tissue of said line to produce an infected embryo;

(b) applying heat shock treatment during said co-cultivation;

(c) culturing the infected embryo to initiate callus on a medium comprising an antibiotic and glucose;

(d) culturing the resulting callus tissue on a medium comprising a selective agent;

(e) selecting transformed callus tissue comprising growing Type II callus; and

(f) regenerating transgenic plants from said Type II callus.